

amendments herein are the same as were made, and accepted by the Patent Office, in previously prosecuted applications using the same specification (divisional applications resulting from the original restriction requirement)..

Claims 41-44 have been replaced by new claims 46-49, respectively, with the changes made as set forth for each claim above. Each of the changes in the claims are primarily to clarify the claim, and it is respectfully submitted that no new matter is added thereby.

In the Office Action of December 19, 1996, the Examiner took the following actions:

(1) stated that the application should be reviewed for errors; (2) stated that the claim numbering had been changed so that claims 67-70 are now claims 41-44 and claim 46 is now claim 45; (3) stated that the application required an abstract; (4) rejected claims 41-45 under the judicially created doctrine of obviousness-type double patenting over claims 1-5 of U.S. Patent No. 5,534253, claim 1 of U.S. Patent No. 5,439,678, claims 1-3 of U.S. Patent No. 5,458,875 and claims 6-8 of U.S. Patent No. 5,480,641; (5) rejected claims 41-45 under 35 U.S.C. § 112, first and second paragraphs, with respect to claim 41 ("storage conditions"; claim 42 ("treating non-Lactobacillus reuteri bacteria", claim 43 ("feeding the animal cells of said strain"), and claim 45 ("A method for reducing the number of bacteria and food item for animals" and "reducing the number of bacteria **more** than does treatment with 250 mM glycerol or glyceraldehyde"); (6) rejected claims 44-45 under 35 U.S.C. § 112, first paragraph with respect to "applying about 200 µg of β-hydroxypropionaldehyde per gram of food item" and "a multi-log factor decrease in the number of any non-*Lactobacillus reuteri* bacteria in any substrate or any anaerobic environment merely if a 10-fold less than the number of bacteria present of *L. reuteri* is added"; and (7) stated that the amendment of the drawings

raises issues of new matter. These rejections are traversed in application to the claims as amended, and consideration is requested of the patentability of claims 45-49 now pending in the application.

(1) Statement that the application should be reviewed for errors

Applicant has reviewed the specification, and has also reviewed the other continuing and divisional applications from the same specification to be sure to find all errors found during review of the specification for those applications, and submits that all known errors have been corrected by amendment.

(2) Statement that the claim numbering had been changed so that claims 67-70 are now claims 41-44 and claim 46 is now claim 45

In this Amendment, claims 41-44, which contain underlining have been replaced with new claims 46-69, because of the provision that claims containing underlining cannot be amended.

(3) Statement that the application required an abstract

Applicant hereby submits the enclosed Abstract, and respectfully submits that this requirement has been met.

(4) Rejection of claims 41-45 under the judicially created doctrine of obviousness-type double patenting over claims 1-5 of U.S. Patent No. 5,534253, claim 1 of U.S. Patent No.

5,439,678, claims 1-3 of U.S. Patent No. 5,458,875 and claims 6-8 of U.S. Patent No. 5,480,641

Although Applicants respectfully submit that there are patentable differences between the instant application and U.S. Patent No. 5,458,875, Applicants submit herein a terminal disclaimer for each of the above-referenced patents to expedite allowability herein. Because one of the cited patents is co-owned by the owner of the instant application and another entity, Applicants have checked with the Patent Office and have been informed that a terminal disclaimer was appropriate.

(5) Rejection of claims 41-45 under 35 U.S.C. § 112, first and second paragraphs, with respect to:

claim 41 ("storage conditions")--Applicant has deleted the adjective "storage" as being unnecessary for the meaning of the claim (new claim 46), and therefore submits new claim 46 replacing claim 41 is patentable under 35 U.S.C. § 112 first and second paragraphs.

claim 42 ("treating non-Lactobacillus reuteri bacteria")--Applicant has replaced this terminology in claim 42 (new claim 47) with "decreasing the numbers of", which is evident from the rest of the claim, and from the disclosure of the specification, and therefore submits new claim 47 replacing claim 42 is patentable under 35 U.S.C. § 112 first and second paragraphs.

claim 43 ("feeding the animal cells of said strain")--Applicant has changed this

copies of the other reference to be forwarded to the Examiner if this would be of assistance in evaluating this matter.

First, there is evidence for receptors in the GI tract for lipid endproducts, i.e., long chain fatty acids and glycerol. These are believed to play a role in regulating emptying GI tract contents into the small intestine. See Melone, J. and Mei, N. 1991. Intestinal effects of the products of lipid digestion on gastric electrical activity in the cat. *Gastroenterology* 100:380-387.

Glycerol is also present in human blood serum. Concentrations varying from 8-20 mg/dl in nonstressed healthy persons are found, although it is not uncommon to find values greater than 75 mg/dl in healthy persons. Elevated blood glycerol levels ranging from 40-250 mg/dl have been reported in patients with various diseases. Also, glycerol is present in interstitial skeletal and adipose tissues at concentrations close to those found in blood. See Howdieshell, T.R. et al. 1995. Effects of free glycerol contained in intravenous fat emulsion on plasma triglyceride determination. *J. Paren. Enteral Nutr.* 19:125-126, and Samra, J.S. et al. 1996. Interstitial glycerol concentration in human skeletal muscle and adipose tissue is close to the concentration in blood. *Clin. Sci.* 90 (copy not enclosed).

Lipid-containing food digestion in the GI tract is an abundant and continuously available source of glycerol. It has been shown, for example, that free glycerol and fatty acids are the final products of human milk fat digestion by the concerted action of gastric lipases, pancreatic colipase-dependent lipase and bile salt-stimulated lipase. See Bernback et al. 1990. The complete digestion of human milk triacylglycerol in vitro requires gastric lipase, pancreatic colipase-dependent lipase, and bile salt-stimulated lipase. *J. Clin. Invest.*

terminology to the terminology suggested by the Examiner: --feeding the animal an amount of Lactobacillus reuteri to colonize--, , and therefore submits new claim 48 replacing claim 43 is patentable under 35 U.S.C. § 112 first and second paragraphs.

With respect to the Examiner's statement regarding sufficiency of disclosure on the number of microorganisms sufficient to colonize, Applicant respectfully submits that one of ordinary skill in the art would find it routine to make such a determination, for example, in fecal samples, using the disclosure of the specific detection method set forth in the specification herein (issued as U.S. Patent No. 5,352,586), and routine experimental methods known in the art. In support of this statement, Applicant respectfully submits the Affidavit of Ivan A. Casas.

With respect to the question of availability of glycerol and/or glyceraldehyde, Applicant respectfully submits that (a) results presented in the specification (e.g, page 12, lines 13-22 and page 15, lines 27-31.) show that glycerol or glyceraldehyde are required for production of this antibiotic, and (b) β -hydroxypropionaldehyde is produced under the conditions of the gastrointestinal tract. Thus, it is clear that glycerol and/or glyceraldehyde must be present in the GI tract.

Furthermore, this conclusion is clearly supported by the literature. There are numerous publications which provide data supporting the conclusion that there are sufficient amounts of glycerol present from both endogenous and exogenous sources, to serve as a substrate for reuterin (β -hydroxypropionaldehyde) production. This conclusion follows from a wide variety of convincing evidence, which is summarized in the following paragraphs. Copies of most of the mentioned references are enclosed; applicant will attempt to obtain

85:1221-1226 (copy not enclosed).

Also, endogenously synthesized gastric mucus, produced by the mucus-secreting epithelial cells, is a viscous and water-insoluble gel that covers the mucosal surface. It is a heterogenous and complex mixture of glycoproteins, lipids, water and electrolytes. The continuous flow and digestive turnover of these mucus secretions provides another source of glycerol for the GI tract. See Dunjic et al. 1993. Green banana protection of gastric mucosa against experimentally induced injuries in rats. Scan. J. Gastroenterol. 28:894-898.

Cell membranes of all prokaryotic microorganisms also contain phospholipids containing the classic triacyglycerol structure. Of the total monosaccharides found in lactobacilli, for example, which are found in relatively high numbers in the monogastric GI tract, glycerol is the most abundant of these monosaccharides. Turnover of lactobacilli and other microorganisms present in the GI tract can contribute yet another source of glycerol to this ecosystem. See Rizzo et al. 1987. Gas chromatography analysis of cellular fatty acids and neutral monosaccharides in the identification of lactobacilli. Appl. Environ. Microbiol. 53:2882-2888.

Finally, it is well known that glycerol is a common endproduct often accumulating in large amounts during growth and metabolism of many bacteria, yeast and fungi, including those found in the GI tracts of humans and other animals. It is believed that glycerol thus produced by gut microorganisms contributes significantly to the pool of free glycerol that is available to other microorganisms in the gut ecosystem, including Lactobacillus reuteri. See, for example, Ciani, M. et al. 1996. Enhanced glycerol content in wines made with immobilized Candida stellata. Appl. Environ. Microbiol. 62:128-132.

claim 45 ("A method for reducing the number of bacteria and food item for animals"
and "reducing the number of bacteria more than does treatment with 250 mM glycerol or
glyceraldehyde"

With respect to the wording of the preamble of claim 45, Applicant respectfully submits that the amendment submitted on October 8, 1996 by telefax is not worded as quoted by the Examiner but has the wording "A method for reducing the number of bacteria in a food item for animals...", not the wording of old claim 49, which this claim replaced (see lines 1-3 of the October 8 amendment).

Applicant has amended claim 45 to add the word --significantly--, and respectfully submits that the actual percentage more is not critical; rather, it is the fact that the claimed invention has a significant result as compared with the mentioned control system, as shown in the specification. Applicant therefore submits that new claim 45 is patentable under 35 U.S.C. § 112, first and second paragraphs.

(6) Rejection of claims 44-45 under 35 U.S.C. § 112, first paragraph with respect to
"applying about 200 µg of β-hydroxypropionaldehyde per gram of food item" and "a multi-
log factor decrease in the number of any non-*Lactobacillus reuteri* bacteria in any substrate
or any anaerobic environment merely if a 10-fold less than the number of bacteria present of
L. reuteri is added"

Support for the terminology on the number of µg of β-hydroxypropionaldehyde is found at page 17, lines 9-12 (4 micrograms of reuterin/5 ml is "one unit"). Thus 50 units is 200 micrograms. Support for the terminology on "per gram of food item" is found at page

20, lines 16-19 and 25-30.

Under the heading of rejection of claims 44-45, the Examiner stated that there was no support for "a multi-log factor decrease" and "ten-fold less". This terminology is not found in either claim 44 or 45 as previously pending herein, but rather is found in claim 42 (now 47). Applicant respectfully submits that support for a multi-log decrease with a 10-fold lower amount of L. reuteri, "under anaerobic conditions and in the presence of glycerol or glyceraldehyde" as set forth in claim 47, is found at page 12, lines 13-35; page 16, lines 14-17; page 17, lines 17-21; page 20, line 29 to page 21, line 1; in Table 8; and in Figures 5, 14, and 15.

(7) Statement that the amendment of the drawings raises issues of new matter.

With respect to Figures 13A and 13B, Applicant respectfully submits that Applicants have amended page 8 at lines 4 and 7 to correct the inadvertent typographic error in describing the two parts of Figure 13. Support for these corrections is clearly found in the specification describing the Figures and in the Figures and figure legends themselves. In particular, the figure labeled "Figure 13A" has the vertical axis simply labeled "CFU ml⁻¹ or PFU ml⁻¹" and the figure labeled "Figure 13B" has the vertical axis labeled the same plus the word "percent" in parentheses. This labeling plus the fact that the largest number on the vertical axis for Figure 13B is "100", while Figure 13A has numbers ranging to "10⁵" clearly shows that the description of Figure 13A as showing "percent" is incorrect, as is the description of Figure 13B as showing "actual counts". Therefore, Applicant has amended to figure legend on page 8 to correctly describe what is shown in these two figures, and has

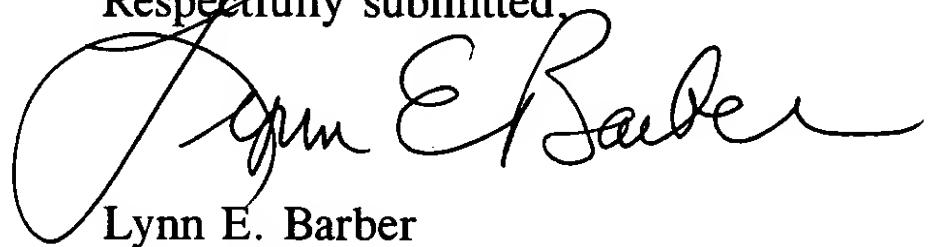
clarified the discussion of these figures on page 17 of the specification.

For all the foregoing reasons, claims 45-49 now pending herein, are submitted to be fully patentably distinguished over the cited references and in allowable condition. Favorable action is therefore requested.

All new claims replace previously pending claims, and there are no new independent claims in this application. It is therefore believed that no fee, except for terminal disclaimer fees and the fee for extension of time, is required for the presentation of this Amendment. Any amounts that may be due for the presentation of this amendment should be charged to Deposit Account No. 02-0825 of Applicant's attorney.

If any questions or issues remain, the resolution of which the Examiner feels would be advanced by a conference (telephonic or personal) with Applicant's attorney, the Examiner is invited to contact such attorney at the telephone number noted below.

Respectfully submitted,



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Enclosures:

- (1) Abstract
- (2) Affidavit of Ivan A. Casas
- (3) Terminal disclaimers
- (4) Petition for Extension of Time and Fee